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NIXON & VANDERHYE, PC
901 NORTH GLEBE ROAD, 11TH FLOOR
ARLINGTON, VA 22203

EXAMINER	
KIM, ALEXANDER D	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/516,338	COSME ET AL.	
	Examiner	Art Unit	
	ALEXANDER D. KIM	1656	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *26 October 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-13 and 21-24* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-13 and 21-24* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *10/26/2007*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☒ Other: *biocompare*.

DETAILED ACTION

Application Status

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 10/26/2007 has been entered.

Applicants requested a suspension with filing of RCE on 10/26/2007. The Examiner requested an interview upon expiration of the suspension as Applicants noted in the reason for the suspension, and Applicants declined to have an interview on 6/24/2008. Thus, the Examiner notified Applicants' representative that the office action would be sent out instead.

Applicants' amendment canceling Claims 14-20; amending Claims 1, 2, 4, 10, 11, 13 and 21-22; adding a new Claim 24 in the paper of 10/26/2007 is acknowledged. Thus, Claims 1-13 and 21-24 are pending in the instant office action.

Information Disclosure Statement

2. The information disclosure statement (IDS) filed on 10/26/2007 has been reviewed, and its references have been considered as shown by the Examiner's initials on the attached copy.

Withdrawn-Objections to the Specification

3. The previous objection to the Abstract is withdrawn by virtue of Applicants' amendment.

Withdrawn-Claim Objections

4. The previous objection of Claim 13 for reciting “further comprising crystallizing” is withdrawn by virtue of Applicants' amendment.

Claim Objections

5. Claim 12 is objected to because of the following informalities:

(a) Claim 12 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. In the instant case, the Claim 12 reciting the limitation "P450 is of SEQ ID NOs: 2, 4, 6 or 8" do not further limit Claim 11 which is also limits the P450 to one of SEQ ID NO: 2, 4, 6 or 8. Appropriate correction is required.

Withdrawn-Claim Rejections - 35 USC § 112

6. The previous rejection of Claims 4-5 under of 35 U.S.C. 112, second paragraph, is withdrawn by virtue of Applicants' amendment.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

7. Claims 10 and 11 are rejected under 35 U.S.C. § 112, first paragraph, written description, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The rejection was stated in the previous office action as it applied to previous Claims 10-11. In response to this rejection, applicants have amended Claims 1, 2, 4, 10, 11, 13 and 21-22, cancelled Claims 14-20, added a new Claim 24; and traverse the rejection as it applies to the newly amended claims.

Applicants argue that instant Claims 10-11 obviates the instant rejection because they are "revised to incorporate the N-terminal modifications and sequences of the proteins of SEQ ID NO: 2, 4, 6 and 8" (see bottom of page 7, Remarks filed on 10/26/2007).

Applicants' arguments have been fully considered but are not deemed persuasive for the following reasons. As disclosed previously, MPEP § 2163 states that a representative number of species means that the species which are adequately

described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus. Thus, as previously noted, the instant specification and the prior art failed to describe a widely varying claimed genus method sufficiently to represent a genus method of purifying P450 having a genus of membrane inserting element, such that the P450 is selected from the group consisting of SEQ ID NO: 2, 4, 6 and 8; wherein said membrane inserting element comprised of unlimited structure having unlimited amino acid residues. The prior art above seem to indicate any residues rich in hydrophobic residues are encompassed in a N-terminal membrane inserting element. However, the instant specification and prior art do not teach the correlation between the structure of N-terminal amino acid sequence to a function of being membrane inserting element. In view of instant amendment, the method of Claim 10 now comprise an N-terminal membrane inserting element (which could be already part of the SEQ ID NO: 2, 4, 6 or 8; or could be further added on to the SEQ ID NO: 2, 4, 6 or 8), whereas the N-terminal membrane inserting element was deleted in the the previous claim. claimed method of purifying the P450 comprise very widely varying any N-terminal inserting element in Claim 10; one skilled in the art would not know the structural feature of very broad structural limitation of comprising any N-terminal membrane inserting element. Also, claimed method purifying the P450 comprising SEQ ID NO: 10 or SEQ ID NO: 11 "in place of the N-terminal inserting element" such that the N-terminal membrane inserting element has to be replaced by SEQ ID NO: 10 or SEQ ID NO: 11; and one skilled in the art would not know how to replace the N-terminal

membrane inserting element because of very widely varing structure of the N-terminal membrane inserting element, which were not sufficiently described be the instant disclosure. The disclosure of deleted sequence (i.e. N-terminal membrane inserting element) by the prior arts and by the instant specification do not describe any structure and functional relationship to predict a structure of other member of the species of any N-terminal membrane inserting element within the full scope of claimed genus method of purification. Thus, for the reasons above, Claims 10 and 11 are rejected.

8. Claims 10 and 11 are rejected under 35 U.S.C. 112, first paragraph, scope of enablement, because the specification, while being enabling for a method for purifying a P450, i.e., SEQ ID NO: 2, 4, 6, or 8; does not reasonably provide enablement for a method for purifying any P450 having any N-terminal membrane inserting element or any N-terminal membrane inserting element of any P450 is replaced by SEQ ID NO: 10 or 8.

The specification does not enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and use of the invention commensurate in scope with these claims.

The rejection was stated in the previous office action as it applied to previous Claims 10-11. In response to this rejection, applicants have amended Claims 1, 2, 4, 10, 11, 13 and 21-22, cancelled Claims 14-20, added a new Claim 24; and traverse the rejection as it applies to the newly amended claims.

Applicants argue that instant Claim 10 obviates the instant rejection because one skilled in the art can make and use the claimed invention, without undue experimentation.

Applicants' arguments have been fully considered but are not deemed persuasive for the following reasons. The breadth of claims encompasses a genus method of purifying P450 (SEQ ID NO: 2, 4, 6 or 8) with any N-terminal membrane inserting element (which has unlimited structure) regardless of having the N-terminal is part of SEQ ID NO: 2, 4, 6 or 8; further added to SEQ ID NO: 2, 4, 6 or 8; or replaced by SEQ ID NO: 10 or 11. The applicants failed to disclose direction or guidance regarding purification of widely varying a P450, wherein any P450 (SEQ ID NO: 2, 4, 6 or 8) comprises very widely varying N-terminal membrane inserting element; comprises further very widely varying N-terminal membrane inserting element to any P450 (SEQ ID NO: 2, 4, 6 or 8); or replacing said N-terminal membrane element in Claim 11. Thus, few examples by the instant specification fails to describe how to make and use the full scope of claimed genus method sufficiently for purifying any P450 (SEQ ID NO: 2, 4, 6 and 8) with or without very widely varying structure of N-terminal membrane inserting element. Because the claimed genus method comprises very widely varying P450 with unlimited structure of N-terminal inserting element, it is unpredictable to determine which sequences should be added or replaced to said SEQ ID NO: 2, 4, 6 and 8 such that one skilled in the art to purify said P450 having (or replacing) any N-terminal membrane binding P450 by the claimed purification method. Thus, the claimed method is unpredictable and undue experimentation is required for one skilled in the art to make

and use the full scope of claimed method. For the reasons above, the instant rejection is maintained.

9. Claim 13 is rejected under 35 U.S.C. 112, first paragraph, written description, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention

The rejection was stated in the previous office action as it applied to previous Claim 13. In response to this rejection, applicants have amended Claims 1, 2, 4, 10, 11, 13 and 21-22, cancelled Claims 14-20, added a new Claim 24; and traverse the rejection as it applies to the newly amended claims.

Applicants argue that instant rejection should be withdrawn because the amended claim referring to the proteins of SEQ ID NO: 2, 4, 6 and 8; and said crystals thereof were in possession at the time of filing the application.

Applicants' arguments have been fully considered but are not deemed persuasive for the following reasons. The instant claim is not drawn to a product (i.e., crystals of protein SEQ ID NO: 2, 4, 6 or 8), but it is drawn to a genus method comprises crystallizing the P450 selected from the group of SEQ ID NO: 2, 4, 6 and 8 by a genus crystallization method, which cannot be adequately described by the disclosure of the instant specification.

The Court of Appeals for the Federal Circuit has recently held that a "written description of an invention involving a chemical genus, like a description of a chemical

species, 'requires a precise definition, such as be structure, formula [or] chemical name,' of the claimed subject matter sufficient to distinguish it from other materials." *University of California v. Eli Lilly and Co.*, 1997 U.S. App. LEXIS 18221, at *23, quoting Fiers v. Revel, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993) (bracketed material in original). To fully describe a genus of genetic material, which is a chemical compound, applicants must (1) fully describe at least one species of the claimed genus sufficient to represent said genus whereby a skilled artisan, in view of the prior art, could predict the structure of other species encompassed by the claimed genus and (2) identify the common characteristics of the claimed molecules, e.g., structure, physical and/or chemical characteristics, functional characteristics when coupled with a known or disclosed correlation between function and structure, or a combination of these (paraphrased from *Enzo Biochemical Inc. v. Gen-Probe Inc.* (CAFC (2002) 63 USPQ2d 1609).

University of Rochester v. G.D. Searle & Co. (69 USPQ2d 1886 (2004)) specifically points to the applicability of both *Lily* and *Enzo Biochemical* to methods of using products, wherein said products lack adequate written description. While in *University of Rochester v. G.D. Searle & Co.* the methods were held to lack written description because not a single example of the product used in the claimed methods was described, the same analysis applies wherein the product, used in the claimed methods, must have adequate written description as noted from *Enzo Biochemical* (see above).

Instant specification describes a method of crystallizing cytochrome P450 2C9 (p. 36), co-crystallization of P450 2C19 (p. 40), crystallization of 2C19-1B (p. 43),

crystallization of P450 2D6 (p. 54) and crystallization of P450 3A4 (p. 60) in examples. However, the specification does not disclose a description of any other P450 crystallization methods that fall within the instant genera of crystallization methods that includes making a crystal of any P450 protein having any space group symmetry, any unit cell dimensions (including error), and any resolution by any method of crystallization. A genus of method crystallizing any P450, as disclosed in Claims cannot be adequately described by the disclosure of the instant specification. The species of instant case do not correlate structure and function from species to genus. Because our understanding of crystallization mechanisms are still incomplete and the factors of macromolecular structure that are involved in crystallization are poorly understood, any method of crystallization encompassed by the breadth of the claims is not adequately described by the representative species of methods of crystallization disclosed in the specification. In general, for a species of crystallization method to be adequately described, the following must be disclosed: a composition of the protein solution and a precipitant solution used in crystallization (exact concentrations, pH and volumes of all molecules used in the crystallization) must be described, including (1) the protein (preferably, with a SEQ ID NO of all included residues) (2) any ligand added (3) the exact contents of precipitant solution. The species of crystallization noted in Examples of the instant specification, as described above, has adequately met this burden. However, description of this single species fails to adequately describe the genus of crystallization methods encompassed by the breadth of the claims.

A singular chemical composition can crystallize differently based on the crystallization conditions, and the space group and unit cell dimensions of a crystal of any given chemical composition can only be determined by analyzing that crystal's X-ray diffraction [Giege *et al*. Crystallogenesis of Biological Macromolecules: Facts and Perspectives. Acta Cryst., (1994) D50: p. 339-350]. Therefore, the suitable crystallization condition(s) disclosed in the specification cannot sufficiently describe a very broad crystallization method, which encompasses any conditions for crystallizing any P450, and one skilled in the art would not be in possession of the claimed genus crystallization methods.

10. Claims 13 is rejected under 35 U.S.C. 112, first paragraph, scope of enablement, because the specification, while being enabling for a method of crystallizing cytochrome P450 2C19 wild type (SEQ ID NO: 2) having space group of P321, cell dimensions of a=158 Å, b=158 Å, c=212 Å and $\alpha=90^{o}$, $\beta=90^{o}$, $\gamma=120^{o}$ (see specification p. 40), and P450 3A4 (SEQ ID NO: 8) having the space group C2 and the unit cell dimensions a=152 Å, b=101 Å, c=78 Å and $\alpha=90^{o}$, $\beta=120^{o}$, $\gamma=90^{o}$ (see specification p. 62); does not reasonably provide enablement for any methods for SEQ ID NO: 2, 4, 6 or 8 crystal preparation comprising any steps as broadly encompassed by the claims.

The rejection was stated in the previous office action as it applied to previous Claim 13. In response to this rejection, applicants have amended Claims 1, 2, 4, 10, 11, 13 and 21-22, cancelled Claims 14-20, added a new Claim 24; and traverse the rejection as it applies to the newly amended claims.

Applicants argue that one skilled in the art will be able to make and use the claimed invention without undue experimentation from the instant teaching, wherein the revised claims refer to the proteins of SEQ ID NO: 2, 4, 6 and 8, crystal of which were in the applicants possession at the time of filing the application.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

The factors to be considered in determining whether undue experimentation is required are summarized *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988). The Court in Wands states: "Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. The key word is 'undue,' not 'experimentation.' " (Wands, 8 USPQ2d 1404). Clearly, enablement of a claimed invention cannot be predicated on the basis of quantity of experimentation required to make or use the invention. "Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." (Wands, 8 USPQ2d 1404). The factors to be considered in determining whether undue experimentation is required include: (1) the quantity of experimentation necessary, (2) the amount or direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or

unpredictability of the art, and (8) the breadth of the claims. While all of these factors are considered, a sufficient amount for a *prima facie* case are discussed below.

The breadth of the claims: Claim 13 is so broad as to encompass a method that comprises making any cytochrome P450 protein crystals in any crystallization condition and/or determining crystal structure of any cytochrome P450 optionally comprising any ligand by X-ray crystallography. The method excludes a said human 2C9 P450 only under salt buffer concentration is 200 to 1000 mM. Method Claim 13 also so broad as to encompass any crystallization method for crystallizing SEQ ID NO: 2, 4, 6 or 8 (cytochrome P450).

The nature of the invention: The invention is drawn to a method that comprises crystallization of cytochrome P450 (SEQ ID NO: 2, 4, 6 or 8) and methods of determining structure coordinate of said protein crystals for a three-dimensional structure determination. At the time of the invention, methods of protein crystallization were well known in the art. However, the ability to crystallize a given protein was, at the least, challenging to a skilled artisan as even minor alterations in the conditions of crystallization could result in altered crystal forms, crystals of sub-diffraction quality, or a lack of crystal growth (as described in further detail below).

The state of the prior art; The level of one of ordinary skill; and The level of predictability in the art: Regarding the claimed crystals and methods thereof, the state of the art at the time of the invention acknowledges a high level of unpredictability for making the full scope of claimed method of crystallization. For example, the reference

of Branden et al. ("Introduction to Protein Structure Second Edition", Garland Publishing Inc., New York, 1999) teaches that "crystallization is usually quite difficult to achieve" (p. 375) and that "well ordered crystals…are difficult to grow because globular protein molecules are large, spherical, or ellipsoidal objects with irregular surfaces, and it is impossible to pack them into a crystal without forming large holes or channels between the individual molecules" (p. 374). Branden et al. further teaches that while there are instances where the structure of a protein has been resolved to a resolution of 1 Å, "only a few small proteins have been determined to such high resolution" (p. 382, first full paragraph). Also, Drenth et al. ("Principles of X-ray Crystallography," Springer, New York, 1995) teach that "the science of protein crystallization is an underdeveloped area" and "protein crystallization is mainly a trial-and-error procedure" (p. 1). One cannot predict *a priori* those conditions that will lead to the successful crystallization of a diffraction-quality crystal as evidenced by Kierzek et al. (2001, *Biophys Chem* 91:1-20), which teaches that "each protein crystallizes under a unique set of conditions that cannot be predicted from easily measurable physico-chemical properties" and that "crystallization conditions must be empirically established for each protein to be crystallized" (p. 2, left column, top). Even minor alterations in the crystallization parameters can affect crystallization as evidenced by Branden et al., which teaches that the formation of protein crystals is critically dependent on a number of different parameters, including pH, temperature, protein concentration, the nature of the solvent and precipitant, as well as the presence of added ions and ligands to the protein (page 375, middle). Branden et al. teaches that even small changes in the crystallization

parameters, *e.g.*, pH, can cause the molecules to pack in different ways to produce different crystal forms (page 374, bottom). Along these same lines, Wiencek (1999, *Ann Rev Biomed Eng* 1:505-534) teaches that "protein solubility will change dramatically as pH is altered by ~ 0.5 pH units…some systems are sensitive to pH changes as small as 0.1 pH units" (p. 514, bottom). In view of these teachings, a skilled artisan would recognize that it is highly unpredictable as to whether diffraction-quality crystals of any cytochrome P450 protein having any sequence can be achieved using the crystallization parameters as set forth in the instant specification. Alternatively, a skilled artisan would recognize that it is highly unpredictable for a method encompassed by the claims as to whether diffraction-quality crystals of any cytochrome P450 protein having any amino acid sequence can be achieved using any crystallization parameters.

The amount of direction provided by the inventor; The existence of working examples: The specification discloses only working examples of crystallizing SEQ ID NO: 2, 4, 6 and 8; and the method of crystallization thereof. Other than these working example(s), the specification fails to provide guidance for altering the crystallization conditions for crystallizing said cytochrome P450 proteins with an expectation of obtaining diffraction-quality crystals. Further, the specification fails to provide guidance for crystallizing said P450 with any P450 binding ligand(s) or any other conditions with an expectation of obtaining diffraction-quality crystals. The instant disclosure has crystals of P450 (SEQ ID NO: 2, 4, 6 and 8). It is unclear if all other crystals would be suitable for structural determination and analysis by the instant specification.

The quantity of experimentation needed to make or use the invention based on the content of the disclosure: While methods of protein crystallization were known at the time of the invention, these methods are specific to a single protein. Thus, a skilled artisan is left to experiment by a trial and error process to determine whether any crystallization conditions can be applied to the crystallization of cytochrome P450 proteins (SEQ ID NO: 2, 4, 6 and 8) optionally bound with any ligand under unlimited different sets of crystallization parameters. In view of the overly broad scope of the claims, the lack of guidance and working examples provided in the specification, the high level of unpredictability as evidenced by the prior art, and the amount of experimentation required to crystallize P450 (SEQ ID NO: 2, 4, 6 and 8) as broadly encompassed by the claims, undue experimentation would be necessary for a skilled artisan to make and use the entire scope of the claimed invention. Thus, applicant has not provided sufficient guidance to enable one skilled in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of crystallization condition of P450 protein (SEQ ID NO: 2, 4, 6 and 8) and having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Withdrawn-Claim Rejections - 35 USC § 102

11. The previous rejection of Claims 1-4, 6-8, 10 and 21-23 are rejected under 35 U.S.C. 102(b) as being anticipated by the reference of Kempf et al. (1995, Archives of Biochemistry and Biophysics, vol. 321, p. 277-288, as cited in the IDS) is withdrawn by virtue of Applicants' argument and in view of reconsideration of the term "Hepes" used by Kempf et al., because Kemp et al. do not teach an evidence that the term "Hepes" is used for the salt thereof (for example, Na Hepes or K Hepes).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

12. Claim 1-4, 6-8 and 21-24 under 35 U.S.C. 103(a) as being unpatentable over Kempf et al. (1995, Archives of Biochemistry and Biophysics, vol. 321, p. 277-288, as cited in the IDS) in view of Sigma-Aldrich product description (see biocompare, Copyright© 1999-2008, in the Attachment).

Claim 1 is drawn to a method for the purification of a cytochrome P450 comprising: (a) expressing in a host cell, (b) recovering the cell from the culture and suspending in a salt buffer having a first salt concentration of 200 to 1000 mM and a conductivity of 12 to 110 mS/cm, (c) lysing the cell in said first salt buffer and removing

cell debris, (d) adding a detergent to the lysate and (e) recovering the P450 from the lysate, wherein the P450 is not a human 2C9 P450 with Pro220 substitution when the salt concentration is 200 to 1000 mM; provided that when said first salt buffer has a concentration of from 200 to 1000mM, the P450 is not a human 2C9 P450 having position 220 substituted by proline. Claims 2-4, 6-8 and 21-24 have additional limitations as disclosed in instant claims.

Kempf et al. teach a method for the purification of His-tagged "N-terminal truncated human cytochrome P450 2D6" ([His]$_6$-**CYP2D6**-Δ25, see bottom of left column, p. 278) and the [His]$_6$-CYP2D6-Δ25 protein as shown in SDS-PAGE gel Figure 3, p. 282, wherein the "N-terminus 25 amino acids --- serve as a membrane anchor" (see Abstract). Kempf et al. teach the method steps comprising: "transformation into E. coli JM109" with "expression plasmid [His]$_6$-CYP2D6-Δ25" (see top of left column, p. 279) and culturing the transformed E. coli as disclosed in "Expression of truncated human P450 2D6" (see middle of left column, p. 279); which meet the limitation of expressing in a cytochrome P450 molecule in a host cell culture in Claim 1(a); the harvested cells were frozen; the frozen cells were thawed and resuspended "in a buffer AD (150 mM NaCl, 0.1 mM EDTA ... 50 mM Hepes, pH 7.8)" (see right column, lines 20-22, on page 279) which meets the limitation of Claim 1(b in part). Kempf et al. teach the cells were disrupted by homogenization and by French press (see right column, lines 23-24, on page 279), and centrifuged, which meets the limitation of lysing cells and removing cell debris in Claim 1(c). The soluble fraction were pooled and C12E9 was added dropwise, which meets the limitation of adding a detergent to a lysate in Claim

1(d). The P450 were recovered by Ni^{2+} -NTA-agarose column (see right column, lines 32-35, on page 279) which meets the limitation of recovering said P450 in Claim 1(e).

Kempf et al. does not teach a method step of suspending said cells in a first salt buffer having a salt concentration of from 200 to 1000 mM and a conductivity of from 12 to 110 mS/cm.

However, one skilled in the art knows that the sodium salt form of Hepes is functional equivalent to a free acid form of Hepes as evidenced by the teaching of disclosure by the "biocompare", which teaches the "buffer solution of HEPES can be prepared by any of several methods including free acid and sodium HEPES.

Therefore, it would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to practice the method of Kempf et al. preparing the HEPES buffer with sodium Hepes with a reasonable expectation of success because they are art recognized equivalents used for the same purpose. According to MPEP 2144.06, “In order to rely on equivalence as a rationale supporting an obviousness rejection, the equivalency must be recognized in the prior art, and cannot be based on applicant’s disclosure or the mere fact that the components at issue are functional or mechanical equivalents.” and “An express suggestion to substitute one equivalent component or process for another is not necessary to render such substitution obvious. In re Fout, 675 F.2d 297, 213”. Because of their art-recognized equivalence, one would have a reasonable expectation of success to use sodium Hepes in place of Hepes free acid, the buffer made by Na Hepes in a method of Kempf et al. would meets the limitation of suspending cells in a first salt buffer having a salt

concentration of 200 mM. Therefore, the claimed invention as a whole was *prima facie* obvious over the combined teachings of the prior art.

The 200 mM salt (i.e., total concentration of salt:150 mM NaCl + 50 mM Na Hepes) would also meets the limitation of "about 500 mM" because the term "about" is broad and it is not defined by the instant application. According to the instant specification page 20, “the buffer comprising a salt which is readily soluble to provide a buffer having a conductivity of from 12 to 110 mS/cm” is “desirably a salt having a concentration in the 200-1000 mM range”, “preferably the salt is a potassium or sodium salt of an anion” and “Potassium phosphate (KPi) is particularly preferred” (see page 20, Salt Buffer). Thus, the buffer AD of Kempf et al. with 5150 mM NaCl, 0.1 mM EDTA ... 50 mM Hepes 200 mM Na salt would have inherent conductivity within the range of 12 to 110 mS/cm. The detergent C12E9 was added dropwise to a final concentration of 0.2%, which meets the limitation of Claim 3. The cytochrome P450 by Kempf et al. is 2D6, which meets the limitation of "not a human 2C9P450 having position 220 substituted by proline", "provided that when said first salt buffer has a concentration of from 200 to 1000mM. Thus, the method of Kempf et al. meets all limitations of Claims 1-3, 6-8 and 22-24.

Kempf et al. also teach the P450 was further purified by applying to a hydroxyl apatite column. The protein P450 in the column was eluted with 200 mM NaPi (which would have conductivity of from 12 to 110 mS/cm as described above). The P450 was dialyzed against buffer H (see bottom of right column, page 279). The dialysis steps meets the limitation of Claim 4 reciting “rapidly desalting” in the Claim 4(f) in view of

broad and reasonable interpretation of the rapid desalting within 30 or 10 minutes. Thus, the method steps of Kempf et al. meet all limitation of Claims 1-4, 6-8 and 21-24.

13. Claims 1-8 and 21-24 under 35 U.S.C. 103(a) as being unpatentable over Kempf et al. (1995, Archives of Biochemistry and Biophysics, vol. 321, p. 277-288, as cited in the IDS) in view of Sigma-Aldrich product description (see biocompare, Copyright© 1999-2008, in the Attachment), in view of Anderson et al. (1968, Journal of Bacteriology, vol. 96, p 93-97).

The rejection was stated in the previous office action as it applied to previous Claim 5. In response to this rejection, applicants have amended Claims 1, 2, 4, 10, 11, 13 and 21-22, cancelled Claims 14-20, added a new Claim 24; and traverse the rejection as it applies to the newly amended claims.

Applicants argue that the Examiner misunderstood the previous statement, which states the instant invention provides for recovery of about 4/5 of the P450, whereas the Kempf results in a loss of about 4/5 of the P450-His6 (see middle of page 11, Remark filed on 10/26/2007). Applicants also argue that there is no indication in either Kempf or Anderson that improvements on this scale could be made.

Applicants' arguments have been fully considered but are not deemed persuasive for the following reasons. Examiner acknowledges "USPTO personnel are to give claims their broadest reasonable interpretation in light of the supporting disclosure" but the "Limitations appearing in the specification but not recited in the

claims should not be read into the claim" (see MPEP 2106[R-5] II). The improvement of purification yield as stated above is not a claim limitation(s).

Applicants also argue that the Examiner has failed to point out any teaching or motivation from Anderson as to why a person of ordinary skill would have modified Kempf to arrive at the method of instant claim 1. As disclosed in the previous 103 rejection the limitation of Claim 1 is met. The instant rejection is focused on a method of using the size exclusion column in Claim 5. However, as disclosed in the previous office action, the motivation was provided by Anderson et al., wherein "The motivation to do so is provided by Anderson et al. who disclose "Gel filtration served simultaneously to desalt and fractionate the product" (see top of page 22 in the final office action mailed out on 9/21/2007).

Applicants argue that the statement in the previous final office action on page 15 is unsupported by reference to any document and provide no substantiated reasons as to why one skilled in the art would have been motivated to perform the process of the instant claim (see top of page 12, Remarks filed on 10/26/2007). It is noted that the said previous statement is disclosed to help applicants what is generally known in the art of protein purification, although the exact purification scheme depends on the protein, in response to the Applicants' argument which reciting what Applicant had discovered. As stated above, the "Limitations appearing in the specification but not recited in the claims should not be read into the claim" (see MPEP 2106[R-5] II). The reference may not be needed for the limitation(s) that is not presented in claim.

The method steps of claims 1-4, 6-8 and 21-24 are disclosed above.

As disclosed in the previous Office Action, Kempf et al. do not teach a method of purification of P450 comprising a step performing a size-exclusion chromatography (limitation of Claim 5) to remove salt.

Anderson et al. teach a method of isolating an enzyme using "gel filtration served simultaneously to desalt and fractionate the product" (see bottom of left column, p. 94).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to practice the method of Kempf et al. purifying N-terminal truncated human P450 2D6 and desalting the purified by using a method step performing size-exclusion chromatography of Anderson et al. instead of dialysis with a reasonable expectation of success to desalt P450 2D6 of Kempf et al. because size-exclusion column separates molecules by the size for the same purpose of dialysis by Kempf et al. The motivation to do so is provided by Anderson et al. who disclose "Gel filtration served simultaneously to desalt and fractionate the product" (see bottom of left column, p. 94). Thus, the gel filtration of Anderson et al. would be advantageous for the purification of a protein over the dialysis of Kempf et al. because, in addition to desalting the protein, gel filtration would provide simultaneous additional purification step. Therefore, the claimed invention as a whole was *prima facie* obvious over the combined teachings of the prior art.

Maintained-Double Patenting

14. The previous provisional rejection of Claims 1-13 and 21-24 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-12

and 21-25 of copending Application No. 10/221,036 is maintained because of Applicants' request to hold the provisional obviousness-type double patenting rejection until such time as allowable subject matter is identified.

The rejection was stated in the previous office action as it applied to previous Claims 1-12 and 21-23. In response to this rejection, applicants have amended Claims 1, 2, 4, 10, 11, 13 and 21-22, cancelled Claims 14-20, added a new Claim 24; and the rejection applies to the newly amended claims.

Conclusion

15. Any inquiry concerning this communication or earlier communications from the examiner should be directed to ALEXANDER D. KIM whose telephone number is (571)272-5266. The examiner can normally be reached on 11AM-7:30PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Kathleen Bragdon can be reached on (571) 272-0931. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic

Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Alexander D Kim/
Examiner, Art Unit 1656

/Richard G Hutson, Ph.D./
Primary Examiner, Art Unit 1652